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THE EFFECT OF LECITHINE ON FAT DEPOSITION IN THE LIVER OF THE NORMAL RAT.

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THE very interesting effects of some component of crude lecithine upon the condition of diabetic animals have been discussed in previous communications from this laboratory [Hershey, 1930; Hershey and Soskin, 1931; Best and Hershey, 1932]. The symptoms exhibited by these animals and the autopsy findings indicate that the characteristic condition is largely attributable to failure of liver function. The results of these studies suggested that an investigation of the effect of lecithine on deposition of fat in the livers of normal animals might be profitable. The literature relevant to this subject has been reviewed in the monographs by Leathes and Raper [1925] and by MacLean and MacLean [1927]. As far as we know, the particular problem in which we are interested has not been investigated by previous workers. The experiments were planned with a view to obtaining deposition of large amounts of fat in the livers of a group of control animals. This was accomplished by feeding a diet high in fairly saturated fats. A second group of animals comparable in every way, as far as we could determine, with the controls, received the same amount of fat as those in the control group and *in addition* varying amounts of crude or purified lecithine. The results demonstrate, among other points, that crude and purified lecithine influence the accumulation of fat in the livers of the test group of animals.

METHODS.

White rats of the Wistar strain, weighing between 150 and 230 g., were used as test animals. Previous to the experiment they had been receiving a stock diet which was adequate in all respects. All the animals used in the experiment were apparently healthy, and great care was taken to ensure that the various groups into which they were divided for any one experiment were as similar as possible. Much time was wasted in preliminary experiments in which attempts were made to use groups of animals in one cage, but this procedure was not found to be feasible. In all the experiments reported in this paper individual cages were used, and notes on the condition of each animal were made throughout the experiment. Every animal in any experiment ate approximately the same amount of the stock diet, which has the following composition: 32·5 p.c. of each of the following—whole cracked wheat, rolled oats and corn meal, and 2·5 p.c. of bone meal. The amounts of fat and of the lecithine under test are given in each of the tables or summaries. In all cases the fat was provided in the form of beef dripping, which has an iodine number of approximately 40. The food was prepared daily. The dripping was melted and added to the stock diet. The mixture was then heated in a boiling water bath for 2 hours, with intermittent stirring. The stirring was continued during the subsequent cooling to ensure the equal distribution of the added fat. The lecithine was thoroughly mixed with the other food by prolonged grinding. The diet for each rat was carefully weighed each day, and in cases where food was left in the cage at the end of 24 hours the amount was determined by weighing. The food trays were designed so that there was little or no food spilled, and the construction of the cages made it possible to recover any spilled food without difficulty. Fat estimations on the food residue showed that the fat had been thoroughly mixed with the other constituents. At the end of the experiment the animals were stunned and decapitated. The animals were not fed on the day on which they were killed. Blood samples were collected from the severed carotid arteries. The livers were removed as soon as the animals were dead, and the content of fatty acid determined by the Leathes and Raper modification of Liebermann's saponification method. The iodine number of the fatty acid of the liver was determined by Wijs' procedure, as outlined by Leathes and Raper. The phospholipines of the liver and blood were calculated from the phosphate content as determined on the alcohol-ether soluble portion by the Briggs' modification [1922] of the Bell-Doisy method. Glycogen was estimated by the Pflüger technique.

EXPERIMENTAL RESULTS.

Egg yolk lecithine. In the first experiment ten rats were used in each of the two groups. The details of the experiments and the results are shown in Tables I and II. The egg yolk lecithine used in these experiments was secured from a commercial firm and was prepared from dried egg yolk, after removal of the fats with benzene, by alcoholic extraction of

TABLE I. Fat diet. Stock diet with fat added (fat 40 p.c. of total food).

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Wt. of liver (g.)	Fatty acids in liver (p.c.)
1	36	99	2·8	170	188	7·77	20·50
2	36	95	2·6	154	167	5·02	5·88
3	35	91	2·6	152	160	4·71	4·20
4	36	94	2·6	178	175	5·58	4·05
5	35	97	2·8	155	167	4·42	5·52
6	36	94	2·6	135	144	4·78	5·12
7	38	100	2·6	121	135	4·51	3·67
8	38	100	2·6	150	144	5·22	6·87
9	38	100	2·6	149	152	6·44	11·30
							Average = 7·36

TABLE II. Fat diet with crude egg lecithine. Stock diet with fat (fat 40 p.c. of stock diet and fat ration) and with lecithine.

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Lecithine daily (g.)	Wt. of liver (g.)	Fatty acids in liver (p.c.)
11	35	102	2·9	181	185	1·4	7·32	3·20
12	36	89	2·5	149	150	1·2	4·57	3·78
13	36	90	2·5	162	157	1·2	6·33	3·38
14	38	103	2·7	193	189	1·3	7·34	3·09
15	38	93	2·4	154	148	1·2	6·86	3·29
16	35	96	2·7	190	182	1·3	7·61	3·30
17	35	91	2·6	144	135	1·3	6·37	3·75
18	38	96	2·5	140	147	1·2	5·48	3·12
19	38	99	2·6	176	170	1·3	6·59	3·97
20	38	108	3·1	185	192	1·5	6·85	3·50
								Average = 3·38

the phospholipines and subsequent removal of the alcohol *in vacuo*. The results of the experiments in which this material was used appear to demonstrate in a convincing manner that some component of lecithine modifies the deposition of fat in the livers of the test animals. The figures also illustrate the very important fact that there is a tremendous variation in the deposition of fat in the livers of different members of a group of animals, each of which is receiving approximately the same amount of fat in the diet.

In most of the experiments all the rats consumed a satisfactory amount of fat. In certain cases, however, the quantity eaten by some of the test animals was not of the same order as that ingested by the controls. The figures for these animals have not been included in the results.

The effects of smaller amounts of lecithine. To study the effects on fatty acids of the liver when small amounts of lecithine were provided, a group of thirty white rats was used. The animals were divided into three groups of ten each, care being taken that the groups were as similar as possible.

Ten rats received 6·15 g. of stock diet *plus* fat 3·5 g. (beef dripping, iodine number 40). Fat formed 40 p.c. of the total food. The experiments lasted 26 days. The average amount of fat taken daily was 3·4 g. The average weight of the rats at the beginning of the experiment was 190 g., and at the end 182 g. The percentage of fatty acids in the livers varied from 7·0 to 25·3 p.c. The average was *15·7* p.c. The iodine numbers varied from 78 to 108. The average was 95. One extraordinarily high iodine number was probably due to an error, and that result is not included in this average.

In the second group all rats received the same amount of stock diet and fat, and in addition egg yolk lecithine. The lecithine formed 9·4 p.c. of the total food. The average figure for the fat eaten daily was 2·97 g., while that for the lecithine was 0·85 g. The average weight of the rats at the beginning of the experiment was 191 g., and the average weight at the end was 177 g. The percentage of fatty acids in the liver varied from 3·3 to 5·1. The average was *3·7* p.c. The iodine number varied from 101 to 136, the average was 129.

The diet for the third group was exactly the same as that of the second, except that lecithine formed 4·9 p.c. of the diet instead of 9·4 p.c. The average figure for the amount of food eaten daily was 3·12 g., while the average figure for the lecithine was 0·44 g. The average figure for the weight at the beginning of the experiment was 191 g., and at the end 187 g. The percentage of fatty acid in the liver varied from 3·1 to 8·2 p.c. The average figure was *4·6* p.c. The iodine numbers varied from 83 to 107. The average figure was 98.

These results appear to furnish evidence that as little as 0·44 g. of crude egg yolk lecithine daily is sufficient to prevent the deposition of large amounts of fat in the liver of a rat under the conditions of these experiments. It will be observed that the rats in the test groups in this series did not eat quite as much added fat as the controls. We have no hesitation in reporting these results, however, for the following reasons: (1) In many other experiments rats from the same colony have invariably shown as high an average fat content of the liver as the test animals in this particular experiment. In these other experiments the controls have eaten even less fat than that taken by the test animals of this experiment. (2) The figures for the individual rats show that many of the animals did eat as much fat as the controls. (3) The test animals ate a certain amount of fatty material in the crude lecithine, which can perhaps be placed to their credit. (4) Furthermore, a study of the tables which contain the results of the determinations made on the control rats demonstrates the fact that the extent of the deposition of liver fat is not proportional to the amount of fat eaten when this amount varies between 2 and 3·5 g. per day. For example, in Table IX the rats which ate 2·3 g. of fat daily showed a much higher fat content than several which ate 2·5 g.

The effects of purified lecithine. Crude egg yolk lecithine was purified by the cadmium chloride procedure of Levene and Rolf [1927]. This fractionation was carried out by one of us (M. E. H.) in the Department of Biochemistry under the direction of Prof. H. D. Kay. The purified lecithine (iodine number 65) obtained was tested on a group of animals.

The results are shown in Tables III and IV. These results establish the fact that purified egg yolk lecithine exerts the same effect as that produced by the crude material.

TABLE III. Fat diet. Stock diet with fat added (fat 40 p.c. of total food).

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Fatty acids in liver (p.c.)	Iodine No.
1	22	54·55	2·48	300	253	6·35	105
2	22	54·55	2·48	202	174	19·2	100
3	22	54·35	2·47	190	194	27·6	—
4	22	54·20	2·46	179	171	19·3	97
5	22	53·60	2·43	189	172	16·2	102
6	20	48·00	2·40	180	177	20·5	95
7	22	54·60	2·48	177	180	15·9	96
8	22	52·90	2·40	169	166	28·2	102
9	22	54·10	2·45	162	160	12·5	104
10	22	53·80	2·44	150	150	17·2	77
						Average = 18·3	

TABLE IV. Fat diet with purified lecithine. Stock diet with fat (fat 40 p.c. of stock diet and fat ration) and with lecithine.

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Lecithine daily (g.)	Fatty acids in liver (p.c.)	Iodine No.
11	22	52·80	2·40	278	257	0·48	3·49	137
12	22	53·60	2·43	203	177	0·49	3·65	107
13	22	49·50	2·35	190	150	0·47	7·4	113
14	22	52·30	2·38	180	171	0·48	3·15	117
15	22	53·00	2·41	199	186	0·48	3·98	118
16	22	46·90	2·13	184	164	0·43	3·58	124
17	22	48·70	2·22	180	166	0·44	3·74	139
18	22	52·80	2·40	170	167	0·48	8·4	76
19	22	51·60	2·34	160	155	0·47	4·05	129
20	22	53·90	2·44	160	151	0·49	4·11	128
							Average = 4·5	

Lecithine from beef liver. In the next series of experiments lecithine, prepared from fresh beef liver, was tested. The lecithine fraction was prepared in the following manner: fresh beef liver was minced and dried in a vacuum oven at low temperature. The dried material was ground and then thoroughly extracted with absolute ethyl alcohol. The material was filtered through paper and the filtrate evaporated to small volume in an efficient vacuum still. The residue was evaporated to a syrup in a large glass flask, and ether was then added until no more material would go into solution. The mixture was allowed to stand overnight in a cold room, and then filtered through paper. The clear ethereal solution was added with stirring to three volumes of acetone. After 48 hours the supernatant fluid was decanted, and the precipitate removed. This material was then stored over paraffin in an atmosphere of nitrogen. This was the material which was subsequently purified by the cadmium chloride procedure. We are indebted to the Connaught Laboratories for the fresh beef liver, for the various reagents, and for the use of the large apparatus required in the preparation of the lecithine.

The effect of crude beef liver lecithine. The results (Tables V and VI) demonstrate that 1 g. and 0·5 g. of lecithine from this source daily are capable of preventing the deposition of fat in the liver of normal white rats.

TABLE V. Fat diet. Stock diet with fat added (fat 40 p.c. of total food).

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Fatty acids in liver (p.c.)	Iodine No.
1	21	52·5	2·5	189	181	29·6	110
2	21	52·5	2·5	171	173	14·5	109
3	21	52·5	2·5	170	172	20·3	116
4	21	52·5	2·5	226	206	25·1	102
5	21	52·5	2·5	164	165	9·2	110
6	22	54·0	2·45	175	167	21·2	100
7	22	55·0	2·5	173	169	16·6	115
8	22	55·0	2·5	160	160	7·8	133
9	22	55·0	2·5	158	154	20·7	63
10	22	55·0	2·5	150	142	7·9	138
					Average	= 17·3	

TABLE VI. Fat diet with crude liver lecithine. Stock diet with fat added (fat 40 p.c. of stock diet and fat ration) and with lecithine.

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Lecithine daily (g.)	Fatty acids in liver (p.c.)	Iodine No.
32	22	52·4	2·38	209	200	0·95	3·1	120
33	22	54·2	2·47	160	168	0·99	4·6	116
34	22	53·7	2·44	172	188	0·97	4·9	175
35	22	51·8	2·36	192	187	0·94	9·5	172
36	22	51·2	2·33	150	143	0·93	4·4	246
37	22	54·2	2·47	151	123	0·99	4·1	192
38	22	51·6	2·35	202	193	0·94	5·1	194
40	22	53·5	2·43	162	175	0·97	3·6	215
41	22	53·2	2·42	170	172	0·48	4·1	205
42	22	55·0	2·50	181	190	0·50	4·0	212
43	22	53·7	2·44	186	192	0·49	5·7	168
44	22	52·5	2·38	165	167	0·48	3·7	213
45	22	53·2	2·42	181	175	0·48	5·7	175

Average 12·4 p.c. lecithine = 4·7
6·7 p.c. lecithine = 4·8

Purified beef liver lecithine. Some 340 g. of purified lecithine (iodine number 75) were obtained by fractionation of the lecithine according to the Levene and Rolf cadmium chloride procedure. Varying amounts of this lecithine were added to the stock diet in the manner described above. The smallest effective daily dose studied was 0·1 g. The results of the experiment in which 0·25 g. was given daily are slightly more consistent, however, and these are given in Tables VII and VIII.

TABLE VII. Fat diet. Stock diet with fat added (fat 40 p.c. of total food).

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Fat excreted daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Fatty acids in liver (p.c.)	Iodine No.
1	20	50·0	2·50	0·17	210	200	9·2	97
2	20	50·0	2·50	0·15	190	184	11·0	95
3	20	50·0	2·50	0·16	170	174	16·0	90
4	20	50·0	2·50	0·15	170	166	8·5	94
5	21	52·5	2·50	0·17	180	176	21·1	—
6	21	52·5	2·50	0·16	176	174	8·2	100
7	21	52·5	2·50	0·16	180	170	11·7	95
8	21	52·5	2·50	0·17	182	170	28·2	97
9	21	51·5	2·46	0·18	166	152	13·8	89
10	21	52·5	2·50	0·14	162	158	16·2	91
						Average =	14·4	

TABLE VIII. Fat diet with purified liver lecithine. Stock diet with fat added (fat 40 p.c. of stock diet and fat ration) and with lecithine.

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Fat excreted daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Lecithine daily (g.)	Fatty acids in liver (p.c.)	Iodine No.
11	21	52·5	2·50	0·17	160	145	0·25	5·30	107
12	21	52·5	2·50	0·20	160	152	0·25	6·55	111
13	21	52·5	2·50	0·18	180	170	0·25	5·85	112
14	21	51·5	2·45	0·19	230	220	0·24	3·87	117
15	21	51·5	2·45	0·18	160	160	0·24	5·85	115
16	21	52·5	2·50	0·15	180	172	0·25	5·40	109
17	21	52·5	2·50	0·15	164	152	0·25	5·08	110
18	21	52·5	2·50	0·15	198	190	0·25	4·27	118
19	21	51·5	2·45	0·16	188	180	0·24	6·12	110
							Average =	5·76	

Proof that the excretion of fatty acids plays no part in the effects of lecithine. Although these results may be shown subsequently to have little physiological significance, they are of considerable interest provided that the effect of lecithine is not due to some relatively unimportant mechanism. The only obvious pitfall seems to be that the lecithine might increase the excretion of fat. In preliminary experiments in which the food residue and fæces were examined for fat, no evidence could be obtained that more fat was lost in the animals on lecithine than in others. In an attempt to make this point perfectly clear, however, the fat in the fæces has been estimated by the saponification method, for each of the members of control and test groups of animals, in three experiments. The fæces were collected daily and were immediately placed in the alkali.

The figures in Tables VII and VIII show that there is no difference in the fat excretion of the control and test groups, and they provide very strong evidence that fat excretion is not a significant factor in the interpretation of these results. In the other experiments in which phospholipines prepared from beef liver and egg yolk lecithine prevented deposition of liver fat, the fat excretion of the animals which received the lecithine was of the same order as that of those on the control diet. The figures for the experiment in which egg yolk lecithine was used are given in Tables IX and X. These results, in addition to the data on fat excretion which they provide, furnish further evidence of the effect of phospholipines derived from egg yolk on deposition of liver fat.

TABLE IX. Fat diet. Stock diet with fat added (fat 40 p.c. of total food).

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Total fat excreted (g.)	Fat excreted daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Fatty acids in liver (p.c.)	Iodine No.
1	21	52·5	2·50	2·46	0·12	171	150	8·9	110
2	21	51·0	2·43	2·19	0·11	155	145	21·5	86
3	21	52·1	2·48	2·74	0·14	158	148	14·8	106
4	21	52·1	2·48	2·67	0·13	200	195	17·3	100
5	21	52·5	2·50	2·49	0·12	174	170	12·3	89
6	21	51·0	2·43	2·69	0·13	165	150	27·7	84
7	21	52·5	2·50	2·38	0·12	163	160	25·0	97
							Average =	18·2	

TABLE X. Fat diet with crude egg lecithine. Stock diet with fat added (fat 40 p.c. of stock diet and fat ration) and with lecithine.

Rat No.	Length of experiment (days)	Total fat eaten (g.)	Fat eaten daily (g.)	Total fat excreted (g.)	Fat excreted daily (g.)	Wt. of rat before (g.)	Wt. of rat after (g.)	Lecithine daily (g.)	Fatty acids in liver (p.c.)	Iodine No.
11	21	51·5	2·43	2·11	0·10	153	150	0·49	3·75	—
12	21	48·7	2·32	1·65	0·08	177	162	0·46	3·50	133
14	21	52·0	2·47	3·16	0·16	181	175	0·49	3·32	140
16	21	50·0	2·38	2·33	0·12	161	137	0·48	3·15	137
20	21	51·6	2·45	2·27	0·11	150	148	0·49	3·50	114
								Average =	3·44	

Lecithine in liver and blood. Determinations of alcohol-ether soluble phosphorus on samples of liver and blood from control and test animals were very kindly made for us by Dr Kay of the Department of Biochemistry. The average liver value, calculated as lecithine, in four control animals was 2·94 p.c. In four test animals each of which ate approximately 0·85 g. of egg yolk lecithine daily the value was 3·38 p.c., and in four test animals which ate 0·44 g. of the same material 3·20 p.c. We are not prepared to conclude without further experiments that the difference between control and test animals is significant, but it is interesting to note that a very large proportion of the total fatty acid in the livers of the test animals must have been derived from the phospholipines. The livers of test animals which contained 3·38 p.c. lecithine showed an average value of 3·85 p.c. fatty acid, those with 3·20 p.c. lecithine 3·80 p.c. fatty acid. The livers of the controls contained 2·94 p.c. lecithine and 11·2 p.c. fatty acids.

The results for the phospholipines, calculated as lecithine, for the blood samples from control animals demonstrate that these values are definitely lower than those obtained from the animals given lecithine. While the animals were not fed on the day on which they were killed, and while we are certain that some of the animals did not eat anything for at least 5 hours previous to their death, we cannot conclude that absorption of lecithine from the intestine did not influence these values obtained for the blood.

Glycogen. Glycogen determinations on samples of the livers of control and test animals have been made for us by our colleague Dr E. T. Waters. Both the fatty livers of the control and the relatively fat-free specimens from the lecithine animals may contain normal quantities of glycogen. The results thus far obtained do not suggest that there is any significant difference in the amounts of liver glycogen in the two groups.

Fatty acid content of whole animal. In several experiments the total fatty acid content of control and test animals has been determined by the saponification procedure. The figures suggest that the animals which had lecithine do not contain as much fatty acid as the controls. The difference is not great, however, and further experiments are required to settle the point. The iodine numbers of the fatty acids obtained from the bodies (livers excluded) of the control and test animals were of the same order.

DISCUSSION.

A review of the literature shows that there is very little information available concerning the effects of diets rich in fat on the deposition of fat in the livers of various species of animals. In the experiments which we carried out during the hot summer weather, difficulties were encountered in securing a high average fat content in the livers of white rats. During the autumn and winter, however, a daily ration containing approximately 2·5 g. of fairly saturated fat has consistently produced a high average fat content in the livers of the control animals (Tables I, III, V, VII and IX). These results are so consistent that it might be considered unnecessary to provide a group of control animals for every new test if one could be absolutely certain that the rats were from the same colony and had received the same diet and attention. It is certain, however, that rats from one colony cannot be used as controls on those from another. In one experiment in which a series of rats from a new colony was used the results had to be discarded because we made the mistake of assuming that the control group would exhibit fatty livers on the same diet as that received by the control rats referred to in the above tables. None of the rats from the new colony which were used as controls had a high liver fat. The cause for this great difference between rats from various colonies would be an interesting subject for further study.

The results of the experiments reported in this paper provide evidence that the addition of crude and purified lecithine from egg yolk and from fresh beef liver to the diet of normal white rats prevents the accumulation of liver fat. These results are very clear-cut. The evidence appears satisfactory that the effect is not due to excretion of fat by the animals on the "lecithine" diet. The fæces were carefully examined by one of us each day, and it appeared probable, even before the actual estimation of the fæcal fat in the control and test groups had been made, that fat excretion by the test animals was not a significant factor in the interpretation of the results. It is in the highest degree unlikely that the saponification method would fail to detect fatty material in one case and not in the other. It is also most improbable that the fat in the intestine of the lecithine-fed animals would be decomposed beyond the fatty acid and glycerol stage.

In the determination of the iodine numbers of the fatty acids from various sources the reagents and the technique have been repeatedly checked by determination of the iodine absorption of a sample of Merck's oleic acid. The iodine number of the beef dripping has ranged between 39 and 41. The tables demonstrate that the iodine number of the fatty acids from the livers of the lecithine-fed animals are on the average considerably higher than those of the control groups. While the average figures may have no great significance in this connection, calculation of these values from the experiments which are reported in this paper shows the following result—control rats 100, lecithine-fed rats 132. The iodine number of the fæcal fat varied between 20 and 50, and was usually between 30 and 40. An interesting point arising out of the determination of the iodine numbers is the very high figures obtained for the fatty acids of the liver of certain of the rats which received lecithine prepared from beef liver. These estimations were made with the same reagents and at the same time as those on the control animals. These high figures suggest that the livers of certain of the animals receiving crude lecithine may contain a large proportion of highly unsaturated fatty acid, but further study is required on this point. If this result can be confirmed however in experiments on larger animals, a good opportunity might be provided for the isolation and identification of the fatty acid or acids responsible.

In an investigation of the effects of the components of lecithine on deposition of liver fat the results of which will be published shortly by two of us (C. H. B. and M. E. H.), evidence has been obtained that the active component of lecithine is choline. In view of this finding a discussion of the physiological significance of the results given in this paper will not be included here.

SUMMARY.

When white rats weighing between 150 and 230 g. receive a daily ration containing 2·5 g. of fairly saturated fat (iodine number approximately 40) for 3 weeks, their livers may be found to contain very large amounts of fatty acids. The average value of the iodine number of these fatty acids in our experiments was approximately 100. Comparable groups of animals receiving the same diet as above, and in addition varying amounts of crude or purified lecithine prepared from egg yolk or beef liver, do not exhibit this increase in liver fat. The values obtained may be as low or lower than those of animals fed on a normal mixed diet. The average value of the iodine numbers of the fatty acids of this group was approximately 132. No evidence could be obtained that increased excretion of fat plays any part in the interpretation of the effect of lecithine.

The results of a preliminary investigation of the effects of lecithine upon liver glycogen and phospholipines, and upon the distribution of fat in other parts of the bodies of the test animals, are briefly discussed.

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